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On October 29, 2001

TOWNSEND and TOWNSEND and CREW LLP

By:

PATENT
Attorney Docket No.: 02307K-059110US
UC Case No. 95-199-1 (cont.)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: WILLIAM M. KAVANAUGH et al. Application No.: 09/894,967 Filed: June 27, 2001 For: BINDING SITES FOR PHOSPHOTYROSINE BINDING DOMAINS	Examiner: Unnassigned Art Unit: Unnassigned COMMUNICATION UNDER 37 C.F.R. §§ 1.821-1.825 AND AMENDMENT

Box SEQUENCE
Assistant Commissioner for Patents
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Sir:

In response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures that accompanied the Notice to file missing parts mailed August 2, 2001, Applicants submit herewith the required paper copy and computer readable copy of the Sequence Listing, pursuant to 37 C.F.R. §§ 1.821-1.825.

IN THE SPECIFICATION:

Please amend the specifications as follows. A marked-up version of all amended paragraphs and claims is attached at the end of this document.

Please replace the paragraph beginning at page 3, line 27, with the following rewritten paragraph:

SUMMARY OF THE INVENTION

The present invention generally provides substantially pure peptides which are capable of binding a binding a PTB domain, wherein the peptide is from 5 to 100 amino acids in length, and comprises a core sequence of amino acids $NX_3X_1X_2X_4$; where X_1 is selected from the group consisting of Y, pY or an analog thereof, E, T, D, Q, A and F; X_2 is selected from pY or an analog thereof, and Y, provided that at least one of X_1 and X_2 is pY, or an analog thereof; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID NO:1). In a preferred embodiment, at least one of X_1 and X_2 will be an analog of phosphotyrosine, and said analog is (phosphonomethyl)-phenylalanine. In preferred aspects, the peptides are from 6 to 100 amino acids in length, and comprise a core sequence of amino acids $X_5NX_3X_1X_2X_4$, wherein X_5 is selected from the group consisting of D, S, E and A (SEQ ID NO:2). In still more preferred peptides, X_2 will be pY. In particularly preferred embodiments, the peptides will be from 6 to 100 amino acids in length, and comprise a core sequence of amino acids selected from the group consisting of $DNX_3X_1pYX_4$ (SEQ ID NO:3) and $ENX_3X_1pYX_4$ (SEQ ID NO:4), where X_4 is selected from the group consisting of W and F.

Please replace the paragraph beginning at page 4, line 11, with the following rewritten paragraph:

Especially preferred peptides will be from 12 to 100 amino acids in length, and which comprise a core sequence of amino acids selected from the group consisting of: AFDNLY(pY)WDQNS (SEQ ID NO:5); AFDNL(pY)YWDQNS (SEQ ID NO:6); and AFDNL(pY)(pY)WDQNS (SEQ ID NO:7). As preferred, are peptides which are from 21 to 100 amino acids in length and which comprise a core sequence of amino acids selected from the group consisting of:

PAFSPAFDNLY(pY)WDQNSSEQG (SEQ ID NO:8); PAFSPAFDNL(pY)YWDQNSSEQG (SEQ ID NO:9); PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10); PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:11); PAFSPAADNL(pY)YWDQNSSEQG (SEQ ID NO:12); PAFSPAADNL(pY)(pY)WDQNSSEQG (SEQ ID NO:13); PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14); PAFSPAFANL(pY)YWDQNSSEQG (SEQ ID NO:15); PAFSPAFANL(pY)(pY)WDQNSSEQG (SEQ ID NO:16); PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17); PAFSPAFSNL(pY)YWDQNSSEQG (SEQ ID NO:18); PAFSPAFSNL(pY)(pY)WDQNSSEQG (SEQ ID NO:19); PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20); PAFSPAFDNA(pY)YWDQNSSEQG (SEQ ID NO:21); PAFSPAFDNA(pY)(pY)WDQNSSEQG (SEQ ID NO:22); PAFSPAFDNLA(pY)WDQNSSEQG (SEQ ID NO:23); PAFSPAFDNLF(pY)WDQNSSEQG (SEQ ID NO:24); PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25); PAFSPAFDNL(pY)YFDQNSSEQG (SEQ ID NO:26); PAFSPAFDNL(pY)(pY)FDQNSSEQG (SEQ ID NO:27); PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28); PAFSPAFDNL(pY)YWAQNSSEQG (SEQ ID NO:29); PAFSPAFDNL(pY)(pY)WAQNSSEQG (SEQ ID NO:30); PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31); PAFSPAFDNL(pY)YWDANSSEQG (SEQ ID NO:32); PAFSPAFDNL(pY)(pY)WDANSSEQG (SEQ ID NO:33); PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34); PAFSPAFDNL(pY)YWDNNSSEQG (SEQ ID NO:35); PAFSPAFDNL(pY)(pY)WDNNSSEQG (SEQ ID NO:36); PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:37); PAFSPAFDNL(pY)YWDDNSSEQG (SEQ ID NO:38); PAFSPAFDNL(pY)(pY)WDDNSSEQG (SEQ ID NO:39); PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:40); PAFSPAFDNL(pY)YWDQASSEQG (SEQ ID NO:41); PAFSPAFDNL(pY)(pY)WDQASSEQG (SEQ ID NO:42); PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43); PAFSPAFDNL(pY)YWDQNASEQG (SEQ ID NO:44); and PAFSPAFDNL(pY)(pY)WDQNASEQG (SEQ ID NO:45).

Please replace the paragraph beginning at page 4, line 37, with the following rewritten paragraph:

In an alternate embodiment, the present invention provides substantially pure peptides which are capable of binding a PTB domain, wherein the peptides are from 21 to about 100 amino acids in length and which comprise a core sequence of amino acids selected from the group consisting of AFGGAVENPE(pY)LAPRAGTASQ (SEQ ID NO:46) and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47).

Please replace the paragraph beginning at page 7, line 15, with the following rewritten paragraph:

Figure 3 is a bar graph showing the effects of various conditions upon PTB domain/phosphopeptide binding. IHA tagged GST-PTB domain fusion protein was incubated in the presence of the following biotinylated peptides: PAFSPAFDNLYYWDQNSSEQG ("b-unphos.") (SEQ ID NO:48); PAFSPAFDNL(pY)(pY)WDQNSSEQG ("b-phos.") (SEQ ID NO:10), alone and in the presence of 100X non-biotinylated, unphosphorylated and phosphorylated peptide ("100X unphos." and "100X phos.", respectively); PAFSPAFDQL(pY)(pY)WDQNSSEQG ("b-N1219Q") (SEQ ID NO:72); and PAFSPAFDDL(pY)(pY)WDQNSSEQG ("b-N1219D") (SEQ ID NO:73). Specific binding was detected using streptavidin-coupled alkaline phosphatase. Also shown is the level of binding by b-phos. and b-unphos. to an SH2 phosphotyrosine binding domain.

Please replace the paragraph beginning at page 12, line 7, with the following rewritten paragraph:

Peptides derived from the c-erbB2 sequence were synthesized, substituting phosphotyrosine for each of the seven tyrosines in the c-erbB2 sequence. These peptides were tested for their ability to compete with c-erbB2 from SKBR3 lysate for binding to PTB domain. The peptides tested and their respective IC_{50} values, are listed in Table 1. The IC_{50} is the concentration of peptide required to inhibit 50 % of normal binding of PTB to c-erbB2.

Table 1

Peptide Sequence	Apparent Inhibition (IC_{50})
PAFSPAFDNL(pY)(pY)WDQNSSEQG ("pY1221/pY1222") (SEQ ID NO:10)	50 nM
AFDNLY(pY)WDQNS ("Y1221/pY1222") (SEQ ID NO:5)	30 nM
AFGGAVENPE(pY)LAPRAGTASQ ("pY1196") (SEQ ID NO:46)	1 μM
EGTPTAENPE(pY)LGLDVPV ("pY1248") (SEQ ID NO:47)	1 μM
APLACSPQPE(pY)VNQPEVRPQS ("pY1139") (SEQ ID NO:49)	>100 μM
SPHDLSPLQR(pY)SEDPTLPL ("pY1112") (SEQ ID NO:50)	>100 μM
TLPLPPETDG(pY)VAPLACSPQ (pY1127") (SEQ ID NO:51)	>100 μM

Please replace the paragraph beginning at page 12, line 26, with the following rewritten paragraph:

The peptides PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10), AFDNLY(pY)WDQNS, AFGGAVENPE(pY)LAPRAGTASQ and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47) showed relatively strong inhibition of PTB domain/c-erbB2 binding with approximate IC_{50}s of 50 nM, 30 nM, 1 μM and 1 μM, respectively. The phosphopeptides SPHDLSPLQR(pY)SEDPTLPL (SEQ ID NO:50), APLACSPQPE(pY)VNQPEVRPQS (SEQ ID NO:49) and TLPLPPETDG(pY)VAPLACSPQ (SEQ ID NO:51), on the other hand appeared to be ineffective.

Please replace the paragraph beginning at page 13, line 1, with the following rewritten paragraph:

Comparison of the sequences of the c-erbB2 derived peptides which were able to bind PTB indicated a common sequence motif of NXX(pY) (SEQ ID NO:52). Furthermore, a similar sequence motif is also found in a number of other signalling proteins associated with cell proliferation, including polyomavirus middle T antigen, the principal transforming protein of the polyomavirus (Campbell, et al., *Proc. Nat'l Acad. Sci. U.S.A.* (1994) 91:6344-6348); Trk tyrosine kinase, associated with signal transduction from nerve growth factors (Obermeier, et al., *J. Biol. Chem.* (1993) 268(31):22963-22966); the EGF receptor (Okabayashi, et al., *J. Biol. Chem.* (1994) 269(28):18674-18678); erbB3, a member of the Type-I (EGF receptor related) family of growth factor receptors (Prigent and Gullick, *EMBO J.* (1994) 13(12):2831-2841); mouse CD3 epsilon chain, integrins and the insulin and IGF receptors. A number of these proteins have been reported to associate with the SHC protein, and the specific sequence motifs are shown in Table 2, below.

Table 2

Protein	Peptide Sequence
Middle T Ag.	LLSNPT(pY)SVMR (SEQ ID NO:53)
erbB3	AFDNPD(pY)WHSRLF (SEQ ID NO:54)

Trk	IENPQ(pY)FSDA (SEQ ID NO:55)
EGF Receptor	SLDNPD(pY)QQDFF (SEQ ID NO:56)

Please replace the paragraph beginning at page 13, line 26, with the following rewritten paragraph:

From the above data, a common PTB recognition sequence, NXXpY (SEQ ID NO:52) is indicated, and more particularly, the motifs NPXpY (SEQ ID NO:57) and NLXpY (SEQ ID NO:58). These sequence motifs appear to be conserved in a variety of signalling proteins, and are present in the peptides which show the greatest affinity for the PTB domain.

Please replace the paragraph beginning at page 13, line 32, with the following rewritten paragraph:

To further characterize the nature of PTB domain binding, peptides were prepared based upon the lead peptide derived from the c-erbB2 protein, PAFSPAFDNL(pY)(pY)WDQNSSEQG ("pY1221/pY1222") (SEQ ID NO:10). These peptides were then tested for their ability to block PTB domain/c-erbB2 binding. The peptides and binding results are shown in Table 3, below.

Table 3

Peptide	Affinity (IC_{50})
PAFSPAFDNLYYWDQNSSEQG ("unphos") (SEQ ID NO:48)	>30μM
PAFSPAFDNL(pS)(pS)WDQNSSEQG ("ser phos") (SEQ ID NO:69)	>30μM
PAFSPAFDNLEEWDQNSSEQG ("glu-glu") (SEQ ID NO:70)	>30μM
PAFSPAFDNLFFWDQNSSEQG ("phe-phe") (SEQ ID NO:71)	>30μM
AFDNL(pY)(pY)WDQNS ("pY1221/pY1222 short")	30nM

(SEQ ID NO:7)	
AFDNL(pY)YWDQNS ("pY1221/Y1222") (SEQ ID NO:6)	1μM
AFDNLY(pY)WDQNS ("Y1221/pY1222") (SEQ ID NO:5)	30nM
DSWDQNQLFS(pY)(pY)SFAPEGPAN (scrambled 1) (SEQ ID NO:59)	>30μM
DSW(pY)SQNQLFDSFAPEG(pY)PAN (scrambled 2) (SEQ ID NO:60)	>30μM

Please replace the paragraph beginning at page 14, line 16, with the following rewritten paragraph:

Peptides in which phosphotyrosine was substituted with phosphoserine or glutamic acid did not compete with c-erbB2 for PTB domain binding (See, also Figure 2, Panel C). Phosphorylated peptide or "phosphopeptide", PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10), which had been dephosphorylated with tyrosine-specific phosphatases, also was unable to block the PTB domain/c-erbB2 interaction. This data demonstrates that the PTB domain specifically recognizes the phosphotyrosine residue.

Please replace the paragraph beginning at page 14, line 25, with the following rewritten paragraph:

The above data indicate that the mere presence of phosphotyrosine alone may not be the only determinant of effective PTB domain binding and competition. The truncated peptide AFDNLY(pY)WDQNS (SEQ ID NO:5), which contained a single phosphotyrosine in the second tyrosine position, had an IC50 approximately equal to that of the double-phosphorylated peptide AFDNL(pY)(pY)WDQNS (SEQ ID NO:7) (See, Figure 2, Panel C). However, the peptide AFDNL(pY)YWDQNS (SEQ ID NO:6), phosphorylated at

only the first tyrosine residue, was 30-fold less effective in competition. While this latter peptide still shows strong inhibition of PTB domain/c-erbB2 interaction, it appears that the PTB domain binds preferentially to phosphotyrosine in the second position. Further, scrambled peptides, which contained the phosphotyrosine residues but a rearranged primary sequence, failed to compete for binding. These data demonstrate that PTB not only binds phosphotyrosine, but also recognizes a range of specific adjacent amino acids.

Please replace the paragraph beginning at page 15, line 8, with the following rewritten paragraph:

Accordingly, to determine which residues in the peptide PAFSPAFDNLY(pY)WDQNSSEQG were important for binding to the PTB domain, a series of peptides containing point mutations in the sequence were prepared and tested for inhibition of PTB domain/c-erbB2 binding. The results are shown in Table 4, below. The substituted residues are underlined. Relative inhibition scales denote IC_{50} values of 50-500 nM ("+++"), 500 nM to 5 μM ("++") 5 to 50 μM ("+") and >50 μM ("-").

Table 4

Peptide	Inhibition
PAFSPA<u>A</u>DNLY(pY)WDQNSSEQG (SEQ ID NO:11)	++
PAFSPAF<u>A</u>NLY(pY)WDQNSSEQG (SEQ ID NO:14)	+
PAFSPAF<u>S</u>NLY(pY)WDQNSSEQG (SEQ ID NO:17)	+
PAFSPAFD<u>A</u>LY(pY)WDQNSSEQG (SEQ ID NO:61)	-
PAFSPAFD<u>Q</u>LY(pY)WDQNSSEQG (SEQ	-

ID NO:62)	
PAFSPAFDDLY(pY)WDQNSSEQG (SEQ ID NO:63)	-
PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20)	++
PAFSPAFDNLAY(pY)WDQNSSEQG (SEQ ID NO:64)	++
PAFSPAFDNLFY(pY)WDQNSSEQG (SEQ ID NO:65)	++
PAFSPAFDNLY(pY)ADQNSSEQG (SEQ ID NO:66)	-
PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25)	++
PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28)	+++
PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31)	++
PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34)	++
PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:67)	++
PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:68)	++
PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43)	++

Please replace the paragraph beginning at page 16, line 6, with the following rewritten paragraph:

From the above data, it can be seen that substitution of the asparagine in the 9th position can have a negative effect on PTB binding. Replacement of aspartic acid in the 8th position also impaired the peptides blocking ability, however this specific residue was not required for competition. Replacement of tryptophan in the 13th position with phenylalanine generally resulted in little loss of affinity, although substitution of this tryptophan with alanine resulted in reduced affinity. This suggests that large hydrophobic or aromatic residues at this position may confer higher affinity. Mutations outside of the central motif DNLY(pY)W (SEQ ID NO:74) generally resulted in only moderate losses in the affinity of the peptide.

Please replace the paragraph beginning at page 16, line 19, with the following rewritten paragraph:

To demonstrate directly that the phosphopeptides bind to the PTB domain, biotinylated peptides were incubated with PTB domain-containing protein ("PTB domain"). The PTB domain was immunoprecipitated and the washed pellet assayed for the presence of bound peptide with streptavidin-coupled alkaline phosphatase. PTB domain was able to bind directly to phosphorylated peptide PAFSPAFDNL(pY)(pY)WDQNSSEQG ("pY1221/pY1222") (SEQ ID NO:10), but did not bind to unphosphorylated peptide (*See* Figure 3). Further, PTB domain did not bind to phosphorylated peptides containing conservative point mutations at the asparagine in the ninth position. The specificity of this sequence for PTB domain was shown by the inability of the SH2 domain of SHC to bind phosphorylated peptide PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10). Additionally, this peptide also blocks association of the SHC PTB domain *in vitro* with pp145, a previously identified target of the SHC protein, derived from activated B cells. *See*, Kavanaugh and Williams, *supra.*

Please replace the paragraph beginning at page 17, line 1, with the following rewritten paragraph:

III. Peptides of the Invention

The peptides of the present invention generally comprise a core sequence which corresponds to a PTB recognition sequence motif. This general PTB recognition sequence motif can be readily identified from the above described data. Typically, the peptides will comprise the sequence motif $NX_3X_1X_2X_4$, where X_1 is Y, pY or an analog thereof, E, T, D, A, F or Q; X_2 is pY or an analog thereof, or Y, provided that at least one of X_1 and X_2 are pY, or an analog thereof; X_3 can be any natural or unnatural amino acid, but is preferably L or A; X_4 is W, F, L, S or Q (SEQ ID NO:1). Generally, this sequence motif may be present as its own peptide, or may be a core of a longer sequence. Generally, the peptides of the present invention will comprise the above motif as a portion or a whole of a peptide of from 5 to about 100 amino acids in length. Typically, the peptides will be from about 6 to about 100 amino acids in length, preferably the peptides will be from about 12 to about 100 amino acids in length, more preferably from about 12 to about 50 amino acids in length, and most preferably, from about 21 to about 50 amino acids in length.

Please replace the paragraph beginning at page 17, line 22, with the following rewritten paragraph:

In particularly preferred aspects of the present invention, the peptides are characterized by the core sequence of amino acids $X_5NX_3X_1X_2X_4$, where X_1, X_2, X_3 and X_4 are as described above, and X_5 can be any natural or unnatural amino acid, but is preferably D, E, S or A (SEQ ID NO:2). Still more preferred are peptides which comprise the core sequence of amino acids $DNX_3X_1pYX_4$ (SEQ ID NO:3) and $ENX_3X_1pYX_4$ (SEQ ID NO:4). The most preferred peptides will generally comprise one of the following core sequences of amino acids:

PAFSPAFDNLY(pY)WDQNSSEQG (SEQ ID NO:8); PAFSPAFDNL(pY)YWDQNSSEQG (SEQ ID NO:9); PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10); AFDNLY(pY)WDQNS (SEQ ID NO:5); AFDNL(pY)YWDQNS (SEQ ID NO:6); AFDNL(pY)(pY)WDQNS (SEQ ID NO:7); PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:11); PAFSPAADNL(pY)YWDQNSSEQG (SEQ ID NO:12);

PAFSPAADNL(pY)(pY)WDQNSSEQG (SEQ ID NO:13);
PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14); PAFSPAFANL(pY)YWDQNSSEQG (SEQ ID NO:15); PAFSPAFANL(pY)(pY)WDQNSSEQG (SEQ ID NO:16);
PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17); PAFSPAFSNL(pY)YWDQNSSEQG (SEQ ID NO:18); PAFSPAFSNL(pY)(pY)WDQNSSEQG (SEQ ID NO:19);
PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20); PAFSPAFDNA(pY)YWDQNSSEQG (SEQ ID NO:21); PAFSPAFDNA(pY)(pY)WDQNSSEQG (SEQ ID NO:22);
PAFSPAFDNLA(pY)WDQNSSEQG (SEQ ID NO:23); PAFSPAFDNLF(pY)WDQNSSEQG (SEQ ID NO:24); PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25);
PAFSPAFDNL(pY)YFDQNSSEQG (SEQ ID NO:26);
PAFSPAFDNL(pY)(pY)FDQNSSEQG (SEQ ID NO:27);
PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28); PAFSPAFDNL(pY)YWAQNSSEQG (SEQ ID NO:29); PAFSPAFDNL(pY)(pY)WAQNSSEQG (SEQ ID NO:30);
PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31); PAFSPAFDNL(pY)YWDANSSEQG (SEQ ID NO:32); PAFSPAFDNL(pY)(pY)WDANSSEQG (SEQ ID NO:33);
PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34); PAFSPAFDNL(pY)YWDNNSSEQG (SEQ ID NO:35); PAFSPAFDNL(pY)(pY)WDNNSSEQG (SEQ ID NO:36);
PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:37); PAFSPAFDNL(pY)YWDDNSSEQG (SEQ ID NO:38); PAFSPAFDNL(pY)(pY)WDDNSSEQG (SEQ ID NO:39);
PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:40); PAFSPAFDNL(pY)YWDQASSEQG (SEQ ID NO:41); PAFSPAFDNL(pY)(pY)WDQASSEQG (SEQ ID NO:42);
PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43);
PAFSPAFDNL(pY)YWDQNASEQG (SEQ ID NO:44);
PAFSPAFDNL(pY)(pY)WDQNASEQG (SEQ ID NO:45);
AFGGAVENPE(pY)LAPRAGTASQ (SEQ ID NO:46) and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47).

Please replace the paragraph beginning at page 21, line 12, with the following rewritten paragraph:

In a preferred aspect of the present invention, the phosphotyrosine (pY) group within the above described peptides can be substituted with an analog of phosphotyrosine which possesses a phosphate group which is nonhydrolyzable, e.g by tyrosine phosphatases. Inclusion of a nonhydrolyzable phosphotyrosine analog allows the peptides of the invention to retain binding and/or inhibitory activity for longer periods of time, in the presence of agents which may remove the phosphate group from the phosphotyrosine, *e.g.*, tyrosine phosphatases, thereby allowing for more effective inhibition and reduced effective amounts, among other benefits. Examples of phosphotyrosine analogs having nonhydrolyzable phosphate groups include, *e.g.*, (phosphonomethyl)phenylalanine ("Pmp"). Pmp is a phosphotyrosine analog in which the >C-O-PO_3H_2 group of pY has been replaced by >C-CH_2-PO_3H_2. Inclusion of this analog within sequences recognized by other phosphotyrosine binding domains yields comparable binding as with their phosphotyrosine-containing counterparts. *See*, Domchek, et al., Biochem. (1992) 31:9865-9870. Thus, in an aspect of the present invention, the peptides of the present invention which comprise a core sequence $NX_3X_1X_2X_4$ (SEQ ID NO:1), where X_1, X_2, X_3 and X_4 are as previously described, the phosphotyrosine residues in X_1 and/or X_2 are substituted with Pmp.

Please replace the paragraph beginning at page 33, line 19, with the following rewritten paragraph:

The direct binding of the phosphorylated peptide PAFSPAFDNL(pY)(pY)WDQNSSEQG ("b-phos.") (SEQ ID NO:10) to the PTB domain is shown in Figure 3. This peptide bound the PTB domain both in the presence and absence of a 100X concentration of unphosphorylated, non-biotinylated peptide. PTB binding was inhibited in the presence of 100X concentration of phosphorylated peptide, which competed for the PTB domain. Unphosphorylated, biotinylated peptide did not bind the PTB domain. Neither the phosphorylated nor unphosphorylated form of this peptide were able to specifically bind to an SH2 domain.

Please replace the paragraph beginning at page 33, line 30, with the following rewritten paragraph:

The peptides PAFSPAFDQL(pY)(pY)WDQNSSEQG ("b-N1219Q") (SEQ ID NO:72) and PAFSPAFDDL(pY)(pY)WDQNSSEQG ("b-N1219D") (SEQ ID NO:73) which carried point mutations in the asparagine residue in the ninth position, also show substantially reduced binding to the PTB domain in these assays (Figure 3).

IN THE CLAIMS

Please amend the following claims:

1. (Amended) A substantially pure peptide which is capable of binding a PTB domain, wherein the peptide is from 5 to 100 amino acids in length, and comprises a core sequence of aminoacids $NX_3X_1X_2X_4$;

wherein X_1 is selected from the group consisting of Y, pY or an analog thereof, E, T, D, Q, A and F;

X_2 is selected from pY or an analog thereof, and Y, provided that at least one of X_1 and X_2 is pY, or an analog thereof;

X_3 is selected from the group consisting of L and A; and

X4 is selected from the group consisting of W, L, S, F and Q (SEQ ID No:1).

2. (Amended) The peptide as recited in claim 1, wherein the peptide is from 6 to 100 amino acids in length, and comprises a core sequence of amino acids $X_5NX_3X_1X_2X_4$, wherein X_5 is selected from the group consisting of D, S, E and A (SEQ ID NO:2).

4. (Amended) The peptide as recited in claim 3, wherein the peptide is from 6 to 100 amino acids in length,. and comprises a core sequence of amino acids selected from the group consisting of $DNX_3X_1pYX_4$ (SEQ ID NO:3) and $ENX_3X_1PYX_4$ (SEQ ID NO:4), where X4 is selected from the group consisting of W and F.

5. (Amended) The peptide as recited in claim 2, wherein the peptide is from 12 to 100 amino acids in length, and comprises a core sequence of amino acids selected from the

group consisting of AFDNLY(pY)WDQNS (SEO ID NO:5), AFDNL(PY)YWDQNS (SEQ ID NO:6) and AFDNL(pY)(pY)WDQNS (SEQ ID NO:7).

6. (Amended) The peptide as recited in claim 2, wherein the peptide is from 21 to 100 amino acids in length, and comprises a core sequence of amino acids selected from the group consisting of: PAFSPAFDNLY(pY)WDQNSSEQG (SEQ ID NO:8); PAFSPAFDNL(pY)YWDQNSSEQG (SEQ ID NO:9); PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10); PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:ll); PAFSPAADNL(pY)YWDQNSSEQG (SEQ ID NO:12); PAFSPAADNL(pY)(pY)WDQNSSEQG (SEQ ID NO:13); PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14); PAFSPAFANL(pY)YWDQNSSEQG (SEQ ID NO:15); PAFSPAFANL(pY)(pY)WDQNSSEQG (SEQ ID NO:16); PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17); PAFSPAFSNL(pY)YWDQNSSEQG (SEQ ID NO:18); PAFSPAFSNL(pY)(pY)WDQNSSEQG (SEQ ID NO:19); PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20); PAFSPAFDNA(pY)YWDQNSSEQG (SEQ ID NO:21); PAFSPAFDNA(pY)(pY)WDQNSSEQG (SEQ ID-NO:22); PAFSPAFDNLA(pY)WDQNSSEQG (SEQ ID NO:23); PAFSPAFDNLF(pY)WDQNSSEQG (SEQ ID NO:24); PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25); PAFSPAFDNL(pY)YFDQNSSEQG (SEQ ID NO:26); PAFSPAFDNL(pY)(pY)FDQNSSEQG (SEQ ID NO:27); PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28); PAFSPAFDNL(pY)YWAQNSSEQG (SEQ ID NO:29); PAFSPAFDNL(pY)(pY)WAQNSSEQG (SEQ ID NO:30); PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31); PAFSPAFDNL(pY)YWDANSSEQG (SEQ ID NO:32); PAFSPAFDNL(pY)(pY)WDANSSEQG (SEQ ID NO:33); PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34); PAFSPAFDNL(pY)YWDNNSSEQG (SEQ ID NO:35); PAFSPAFDNL(pY)(pY)WDNNSSEQG (SEQ ID NO:36); PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:37); PAFSPAFDNL(pY)YWDDNSSEQG (SEQ ID NO:38); PAFSPAFDNL(pY)(pY)WDDNSSEQG (SEQ ID NO:39); PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:40); PAFSPAFDNL(PY)YWDQASSEQG

(SEQ ID NO:41); PAFSPAFDNL(pY)(pY)WDQASSEQG (SEQ ID NO:42); PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43); PAFSPAFDNL(pY)YWDQNASEQG (SEQ ID NO:44); and PAFSPAFDNL(pY)(pY)WDQNASEQG (SEQ ID NO:45).

8. (Amended) A substantially pure peptide which is capable of binding a PTB domain, wherein the peptide is from 21 to about loo amino acids in length and which comprises a core sequence of amino acids selected from the group consisting of AFGGAVENPE(pY)LAPRAGTASQ (SEQ ID NO:46) and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47).

10. (Amended) A method of determining whether a protein comprises a PTB domain, comprising the steps of:

contacting the protein with a peptide, which peptide is from 5 to 100 amino acids in length and comprises a core sequence of amino acids $NX_3X_1X_2X_4$, wherein X_1 is selected from the group consisting of Y, pY, E, T, D, Q, A and F; X_2 is selected from pY and Y, provided that at least one of X_1 and X_2 is pY; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID NO:l); and determining whether the peptide binds to the protein during said contacting step, where the binding of the peptide to the protein is indicative that the protein comprises a PTB domain.

13. (Amended) A method of determining whether a test compound is an agonist or antagonist of a PTB/phosphorylated ligand interaction, comprising the steps of:

incubating the test compound with a protein comprising a PTB domain and a peptide, which peptide is from 5 to 100 amino acids in length and which comprises a core amino acid sequence $NX_3X_1X_2X_4$, wherein X_1 is selected f rom the group consisting of Y, pY, E, T, D, Q, A and F; X_2 is selected from pY and Y, provided that at least one of X_1 and X_2 is pY; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID NO:l); and

determining the amount of protein bound to the peptide during said incubating step; and

comparing the amount of protein bound to the peptide during said incubating step to an amount of protein bound to the peptide in the absence of the test compound, the increase or decrease in the amount of protein bound to the peptide in the presence of the test compound being indicative that the test compound is an agonist or antagonist of PTB domain/phosphorylate ligand interaction, respectively.

17. (Amended) A method of obtaining substantially pure PTB-domain-containing protein from a mixture of different proteins, comprising the steps of:

providing a peptide which is from 5 to 100 amino acids in length, and which comprises a core amino acid sequence $NX_3X_1X_2X_4$, wherein X_1 is selected from the group consisting of Y, pY, E, T, D, Q, A and F; X_2 is selected from pY and Y, provided that at least one of X_1 and X_2 is pY; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID-NO:l); bound to a solid support;

contacting the mixture of different proteins with the peptide bound to the solid support whereby the PTB domain-containing protein is bound to the peptide;

washing the solid support to remove unbound proteins;

and

eluting substantially pure PTB-domain-containing protein from the solid support.

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 33, at the end of the application.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-74, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

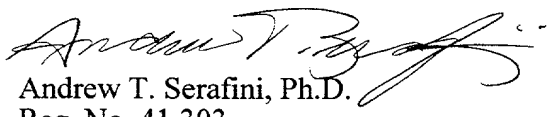
The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

In addition. Applicants have corrected a number of typographical errors in the present application.

Applicants believe that no fee is required for this submission. However, if a fee is required, the Commissioner is authorized to deduct such fee from the undersigned's Deposit Account No. 20-1430. Please deduct any additional fees from, or credit any overpayment to, the above-noted Deposit Account.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 326-2400, extension 5209.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 3, line 27, has been amended as follows:

SUMMARY OF THE INVENTION

The present invention generally provides substantially pure peptides which are capable of binding a binding a PTB domain, wherein the peptide is from 5 to 100 amino acids in length, and comprises a core sequence of amino acids $NX_3X_1X_2X_4$; where X_1 is selected from the group consisting of Y, pY or an analog thereof, E, T, D, Q, A and F; X_2 is selected from pY or an analog thereof, and Y, provided that at least one of X_1 and X_2 is pY, or an analog thereof; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID NO:1). In a preferred embodiment, at least one of X_1 and X_2 will be an analog of phosphotyrosine, and said analog is (phosphonomethyl)-phenylalanine. In preferred aspects, the peptides are from 6 to 100 amino acids in length, and comprise a core sequence of amino acids $X_5NX_3X_1X_2X_4$, wherein X_5 is selected from the group consisting of D, S, E and A (SEQ ID NO:2). In still more preferred peptides, X_2 will be pY. In particularly preferred embodiments, the peptides will be from 6 to 100 amino acids in length, and comprise a core sequence of amino acids selected from the group consisting of $DNX_3X_1pYX_4$ (SEQ ID NO:3) and $ENX_3X_1pYX_4$ (SEQ ID NO:4), where X_4 is selected from the group consisting of W and F.

Paragraph beginning at page 4, line 11, has been amended as follows:

Especially preferred peptides will be from 12 to 100 amino acids in length, and which comprise a core sequence of amino acids selected from the group consisting of: AFDNLY(pY)WDQNS (SEQ ID NO:5); AFDNL(pY)YWDQNS (SEQ ID NO:6); and AFDNL(pY)(pY)WDQNS (SEQ ID NO:7). As preferred, are peptides which are from 21 to 100 amino acids in length and which comprise a core sequence of amino acids selected from the group consisting of:

PAFSPAFDNLY(pY)WDQNSSEQG (SEQ ID NO:8); PAFSPAFDNL(pY)YWDQNSSEQG (SEQ ID NO:9); PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10); PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:11); PAFSPAADNL(pY)YWDQNSSEQG (SEQ ID NO:12); PAFSPAADNL(pY)(pY)WDQNSSEQG (SEQ ID NO:13); PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14); PAFSPAFANL(pY)YWDQNSSEQG (SEQ ID NO:15); PAFSPAFANL(pY)(pY)WDQNSSEQG (SEQ ID NO:16); PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17); PAFSPAFSNL(pY)YWDQNSSEQG (SEQ ID NO:18); PAFSPAFSNL(pY)(pY)WDQNSSEQG (SEQ ID NO:19); PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20); PAFSPAFDNA(pY)YWDQNSSEQG (SEQ ID NO:21); PAFSPAFDNA(pY)(pY)WDQNSSEQG (SEQ ID NO:22); PAFSPAFDNLA(pY)WDQNSSEQG (SEQ ID NO:23); PAFSPAFDNLF(pY)WDQNSSEQG (SEQ ID NO:24); PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25); PAFSPAFDNL(pY)YFDQNSSEQG (SEQ ID NO:26); PAFSPAFDNL(pY)(pY)FDQNSSEQG (SEQ ID NO:27); PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28); PAFSPAFDNL(pY)YWAQNSSEQG (SEQ ID NO:29); PAFSPAFDNL(pY)(pY)WAQNSSEQG (SEQ ID NO:30); PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31); PAFSPAFDNL(pY)YWDANSSEQG (SEQ ID NO:32); PAFSPAFDNL(pY)(pY)WDANSSEQG (SEQ ID NO:33); PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34); PAFSPAFDNL(pY)YWDNNSSEQG (SEQ ID NO:35); PAFSPAFDNL(pY)(pY)WDNNSSEQG (SEQ ID NO:36); PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:37); PAFSPAFDNL(pY)YWDDNSSEQG (SEQ ID NO:38); PAFSPAFDNL(pY)(pY)WDDNSSEQG (SEQ ID NO:39);

PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:40); PAFSPAFDNL(pY)YWDQASSEQG (SEQ ID NO:41); PAFSPAFDNL(pY)(pY)WDQASSEQG (SEQ ID NO:42); PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43); PAFSPAFDNL(pY)YWDQNASEQG (SEQ ID NO:44); and PAFSPAFDNL(pY)(pY)WDQNASEQG (SEQ ID NO:45).

Paragraph beginning at page 4, line 37, has been amended as follows:

In an alternate embodiment, the present invention provides substantially pure peptides which are capable of binding a PTB domain, wherein the peptides are from 21 to about 100 amino acids in length and which comprise a core sequence of amino acids selected from the group consisting of AFGGAVENPE(pY)LAPRAGTASQ (SEQ ID NO:46) and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47).

Paragraph beginning at page 7, line 15, has been amended as follows:

Figure 3 is a bar graph showing the effects of various conditions upon PTB domain/phosphopeptide binding. IHA tagged GST-PTB domain fusion protein was incubated in the presence of the following biotinylated peptides: PAFSPAFDNLYYWDQNSSEQG ("b-unphos.") (SEQ ID NO:48); PAFSPAFDNL(pY)(pY)WDQNSSEQG ("b-phos.") (SEQ ID NO:10), alone and in the presence of 100X non-biotinylated, unphosphorylated and phosphorylated peptide ("100X unphos." and "100X phos.", respectively); PAFSPAFDQL(pY)(pY)WDQNSSEQG ("b-N1219Q") (SEQ ID NO:72); and PAFSPAFDDL(pY)(pY)WDQNSSEQG ("b-N1219D") (SEQ ID NO:73). Specific binding was detected using streptavidin-coupled alkaline phosphatase. Also shown is the level of binding by b-phos. and b-unphos. to an SH2 phosphotyrosine binding domain.

Paragraph beginning at page 12, line 7, has been amended as follows:

Peptides derived from the c-erbB2 sequence were synthesized, substituting phosphotyrosine for each of the seven tyrosines in the c-erbB2 sequence. These peptides were tested for their ability to compete with c-erbB2 from SKBR3 lysate for binding to PTB domain. The peptides tested and their respective IC_{50} values, are listed in Table 1. The IC_{50} is the concentration of peptide required to inhibit 50 % of normal binding of PTB to c-erbB2.

Table 1

Peptide Sequence	Apparent Inhibition (IC_{50})
PAFSPAFDNL(pY)(pY)WDQNSSEQG ~~("Y1221/pY1222")~~ ("pY1221/pY1222") (SEQ ID NO:10)	50 nM
AFDNLY(pY)WDQNS ("Y1221/pY1222") (SEQ ID NO:5)	30 nM
AFGGAVENPE(pY)LAPRAGTASQ ~~("pY1196")~~ ("pY1196") (SEQ ID NO:46)	1 μM
EGTPTAENPE(pY)LGLDVPV ("pY1248") (SEQ ID NO:47)	1 μM
APLACSPQPE(pY)VNQPEVRPQS ("pY1139") (SEQ ID NO:49)	>100 μM
SPHDLSPLQR(pY)SEDPTLPL ("pY1112") (SEQ ID NO:50)	>100 μM
TLPLPPETDG(pY)VAPLACSPQ (pY1127") (SEQ ID NO:51)	>100 μM

Paragraph beginning at page 12, line 26, has been amended as follows:

The peptides PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10), AFDNLY(pY)WDQNS, AFGGAVENPE(pY)LAPRAGTASQ and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47) showed relatively strong inhibition of PTB domain/c-erbB2 binding with approximate IC_{50}s of 50 nM, 30 nM, 1 μM and 1 μM, respectively. The phosphopeptides ~~SPHDLSPLQR(pY)SEDPTLP~~ SPHDLSPLQR(pY)SEDPTLPL (SEQ ID NO:50), APLACSPQPE(pY)VNQPEVRPQS (SEQ ID NO:49) and TLPLPPETDG(pY)VAPLACSPQ (SEQ ID NO:51), on the other hand appeared to be ineffective.

Paragraph beginning at page 13, line 1, has been amended as follows:

Comparison of the sequences of the c-erbB2 derived peptides which were able to bind PTB indicated a common sequence motif of NXX(pY) (SEQ ID NO:52). Furthermore, a similar sequence motif is also found in a number of other signalling proteins associated with cell proliferation, including polyomavirus middle T antigen, the principal transforming protein of the polyomavirus (Campbell, et al., *Proc. Nat'l Acad. Sci. U.S.A.* (1994) 91:6344-6348); Trk tyrosine kinase, associated with signal transduction from nerve growth factors (Obermeier, et al., *J. Biol. Chem.* (1993) 268(31):22963-22966); the EGF receptor (Okabayashi, et al., *J. Biol. Chem.* (1994) 269(28):18674-18678); erbB3, a member of the Type-I (EGF receptor related) family of growth factor receptors (Prigent and Gullick, *EMBO J.* (1994) 13(12):2831-2841); mouse CD3 epsilon chain, integrins and the insulin and IGF receptors. A number of these proteins have been reported to associate with the SHC protein, and the specific sequence motifs are shown in Table 2, below.

Table 2

Protein	Peptide Sequence
Middle T Ag.	LLSNPT(pY)SVMR (SEQ ID NO:53)
erbB3	AFDNPD(pY)WHSRLF (SEQ ID NO:54)

Trk	IENPQ(pY)FSDA (SEQ ID NO:55)
EGF Receptor	SLDNPD(pY)QQDFF (SEQ ID NO:56)

Paragraph beginning at page 13, line 26, has been amended as follows:

From the above data, a common PTB recognition sequence, NXXpY (SEQ ID NO:52) is indicated, and more particularly, the motifs NPXpY (SEQ ID NO:57) and NLXpY (SEQ ID NO:58). These sequence motifs appear to be conserved in a variety of signalling proteins, and are present in the peptides which show the greatest affinity for the PTB domain.

Paragraph beginning at page 13, line 32, has been amended as follows:

To further characterize the nature of PTB domain binding, peptides were prepared based upon the lead peptide derived from the c-erbB2 protein, PAFSPAFDNL(pY)(pY)WDQNSSEQG ("pY1221/pY1222") (SEQ ID NO:10). These peptides were then tested for their ability to block PTB domain/c-erbB2 binding. The peptides and binding results are shown in Table 3, below.

Table 3

Peptide	Affinity (IC_{50})
PAFSPAFDNLYYWDQNSSEQG ("unphos") (SEQ ID NO:48)	>30μM
PAFSPAFDNL(pS)(pS)WDQNSSEQG ("ser phos") (SEQ ID NO:69)	>30μM
PAFSPAFDNLEEWDQNSSEQG ("glu-glu") (SEQ ID NO:70)	>30μM
PAFSPAFDNLFFWDQNSSEQG ("phe-phe") (SEQ ID NO:71)	>30μM
AFDNL(pY)(pY)WDQNS ("pY1221/pY1222 short") (SEQ ID NO:7)	30nM
AFDNL(pY)YWDQNS ("pY1221/Y1222") (SEQ ID	1μM

NO:6)	
AFDNLY(pY)WDQNS ("Y1221/pY1222") (SEQ ID NO:5)	30nM
DSWDQNQLFS(pY)(pY)SFAPEGPAN (scrambled 1) (SEQ ID NO:59)	>30μM
DSW(pY)SQNQLFDSFAPEG(pY)PAN (scrambled 2) (SEQ ID NO:60)	>30μM

Paragraph beginning at page 14, line 16, has been amended as follows:

Peptides in which phosphotyrosine was substituted with phosphoserine or glutamic acid did not compete with c-erbB2 for PTB domain binding (See, also Figure 2, Panel C). Phosphorylated peptide or "phosphopeptide", PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10), which had been dephosphorylated with tyrosine-specific phosphatases, also was unable to block the PTB domain/c-erbB2 interaction. This data demonstrates that the PTB domain specifically recognizes the phosphotyrosine residue.

Paragraph beginning at page 14, line 25, has been amended as follows:

The above data indicate that the mere presence of phosphotyrosine alone may not be the only determinant of effective PTB domain binding and competition. The truncated peptide AFDNLY(pY)WDQNS (SEQ ID NO:5), which contained a single phosphotyrosine in the second tyrosine position, had an IC50 approximately equal to that of the double-phosphorylated peptide AFDNL(pY)(pY)WDQNS (SEQ ID NO:7) (See, Figure 2, Panel C). However, the peptide AFDNL(pY)YWDQNS (SEQ ID NO:6), phosphorylated at only the first tyrosine residue, was 30-fold less effective in competition. While this latter peptide still shows strong inhibition of PTB domain/c-erbB2 interaction, it appears that the PTB domain binds preferentially to phosphotyrosine in the second position. Further, scrambled peptides, which contained the phosphotyrosine residues but a rearranged primary

sequence, failed to compete for binding. These data demonstrate that PTB not only binds phosphotyrosine, but also recognizes a range of specific adjacent amino acids.

Paragraph beginning at page 15, line 8, has been amended as follows:

Accordingly, to determine which residues in the peptide PAFSPAFDNLY(pY)WDQNSSEQG were important for binding to the PTB domain, a series of peptides containing point mutations in the sequence were prepared and tested for inhibition of PTB domain/c-erbB2 binding. The results are shown in Table 4, below. The substituted residues are underlined. Relative inhibition scales denote IC_{50} values of 50-500 nM ("+++"), 500 nM to 5 μM ("++") 5 to 50 μM ("+") and >50 μM ("-").

Table 4

Peptide	Inhibition
PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:11)	++
PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14)	+
PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17)	+
PAFSPAFDALY(pY)WDQNSSEQG (SEQ ID NO:61)	-
PAFSPAFDQLY(pY)WDQNSSEQG (SEQ ID NO:62)	-
PAFSPAFDDLY(pY)WDQNSSEQG (SEQ ID NO:63)	-

PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20)	++
PAFSPAFDNLAY(pY)WDQNSSEQG (SEQ ID NO:64)	++
PAFSPAFDNLFY(pY)WDQNSSEQG (SEQ ID NO:65)	++
PAFSPAFDNLY(pY)ADQNSSEQG (SEQ ID NO:66)	-
PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25)	++
PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28)	+++
PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31)	++
PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34)	++
PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:67)	++
PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:68)	++
PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43)	++

Paragraph beginning at page 16, line 6, has been amended as follows:

From the above data, it can be seen that substitution of the asparagine in the 9th position can have a negative effect on PTB binding. Replacement of aspartic acid in the 8th position also impaired the peptides blocking ability, however this specific residue was not required for competition. Replacement of tryptophan in the 13th position with phenylalanine generally resulted in little loss of affinity, although substitution of this tryptophan with alanine resulted in reduced affinity. This suggests that large hydrophobic or aromatic residues at this position may confer higher affinity. Mutations outside of the central motif DNLY(pY)W (SEQ ID NO:74) generally resulted in only moderate losses in the affinity of the peptide.

Paragraph beginning at page 16, line 19, has been amended as follows:

To demonstrate directly that the phosphopeptides bind to the PTB domain, biotinylated peptides were incubated with PTB domain-containing protein ("PTB domain"). The PTB domain was immunoprecipitated and the washed pellet assayed for the presence of bound peptide with streptavidin-coupled alkaline phosphatase. PTB domain was able to bind directly to phosphorylated peptide PAFSPAFDNL(pY)(pY)WDQNSSEQG ("pY1221/pY1222") (SEQ ID NO:10), but did not bind to unphosphorylated peptide (*See* Figure 3). Further, PTB domain did not bind to phosphorylated peptides containing conservative point mutations at the asparagine in the ninth position. The specificity of this sequence for PTB domain was shown by the inability of the SH2 domain of SHC to bind phosphorylated peptide PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10). Additionally, this peptide also blocks association of the SHC PTB domain *in vitro* with pp145, a previously identified target of the SHC protein, derived from activated B cells. *See*, Kavanaugh and Williams, *supra.*

Paragraph beginning at page 17, line 1, has been amended as follows:

III. Peptides of the Invention

The peptides of the present invention generally comprise a core sequence which corresponds to a PTB recognition sequence motif. This general PTB recognition sequence motif can be

readily identified from the above described data. Typically, the peptides will comprise the sequence motif $NX_3X_1X_2X_4$, where X_1 is Y, pY or an analog thereof, E, T, D, A, F or Q; X_2 is pY or an analog thereof, or Y, provided that at least one of X_1 and X_2 are pY, or an analog thereof; X_3 can be any natural or unnatural amino acid, but is preferably L or A; X_4 is W, F, L, S or Q (SEQ ID NO:1). Generally, this sequence motif may be present as its own peptide, or may be a core of a longer sequence. Generally, the peptides of the present invention will comprise the above motif as a portion or a whole of a peptide of from 5 to about 100 amino acids in length. Typically, the peptides will be from about 6 to about 100 amino acids in length, preferably the peptides will be from about 12 to about 100 amino acids in length, more preferably from about 12 to about 50 amino acids in length, and most preferably, from about 21 to about 50 amino acids in length.

Paragraph beginning at page 17, line 22, has been amended as follows:

In particularly preferred aspects of the present invention, the peptides are characterized by the core sequence of amino acids $X_5NX_3X_1X_2X_4$, where X_1, X_2, X_3 and X_4 are as described above, and X_5 can be any natural or unnatural amino acid, but is preferably D, E, S or A (SEQ ID NO:2). Still more preferred are peptides which comprise the core sequence of amino acids $DNX_3X_1pYX_4$ (SEQ ID NO:3) and $ENX_3X_1pYX_4$ (SEQ ID NO:4). The most preferred peptides will generally comprise one of the following core sequences of amino acids:

PAFSPAFDNLY(pY)WDQNSSEQG (SEQ ID NO:8); PAFSPAFDNL(pY)YWDQNSSEQG (SEQ ID NO:9); PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10); AFDNLY(pY)WDQNS (SEQ ID NO:5); AFDNL(pY)YWDQNS (SEQ ID NO:6); AFDNL(pY)(pY)WDQNS (SEQ ID NO:7); PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:11); PAFSPAADNL(pY)YWDQNSSEQG (SEQ ID NO:12); PAFSPAADNL(pY)(pY)WDQNSSEQG (SEQ ID NO:13); PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14); PAFSPAFANL(pY)YWDQNSSEQG (SEQ ID NO:15); PAFSPAFANL(pY)(pY)WDQNSSEQG (SEQ ID NO:16); PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17); PAFSPAFSNL(pY)YWDQNSSEQG

(SEQ ID NO:18); PAFSPAFSNL(pY)(pY)WDQNSSEQG (SEQ ID NO:19); PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20); PAFSPAFDNA(pY)YWDQNSSEQG (SEQ ID NO:21); PAFSPAFDNA(pY)(pY)WDQNSSEQG (SEQ ID NO:22); PAFSPAFDNLA(pY)WDQNSSEQG (SEQ ID NO:23); PAFSPAFDNLF(pY)WDQNSSEQG (SEQ ID NO:24); PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25); PAFSPAFDNL(pY)YFDQNSSEQG (SEQ ID NO:26); PAFSPAFDNL(pY)(pY)FDQNSSEQG (SEQ ID NO:27); PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28); PAFSPAFDNL(pY)YWAQNSSEQG (SEQ ID NO:29); PAFSPAFDNL(pY)(pY)WAQNSSEQG (SEQ ID NO:30); PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31); PAFSPAFDNL(pY)YWDANSSEQG (SEQ ID NO:32); PAFSPAFDNL(pY)(pY)WDANSSEQG (SEQ ID NO:33); PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34); PAFSPAFDNL(pY)YWDNNSSEQG (SEQ ID NO:35); PAFSPAFDNL(pY)(pY)WDNNSSEQG (SEQ ID NO:36); PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:37); PAFSPAFDNL(pY)YWDDNSSEQG (SEQ ID NO:38); PAFSPAFDNL(pY)(pY)WDDNSSEQG (SEQ ID NO:39); PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:40); PAFSPAFDNL(pY)YWDQASSEQG (SEQ ID NO:41); PAFSPAFDNL(pY)(pY)WDQASSEQG (SEQ ID NO:42); PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43); PAFSPAFDNL(pY)YWDQNASEQG (SEQ ID NO:44); PAFSPAFDNL(pY)(pY)WDQNASEQG (SEQ ID NO:45); AFGGAVENPE(pY)LAPRAGTASQ (SEQ ID NO:46) and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47).

Paragraph beginning at page 21, line 12, has been amended as follows:

In a preferred aspect of the present invention, the phosphotyrosine (pY) group within the above described peptides can be substituted with an analog of phosphotyrosine which possesses a phosphate group which is nonhydrolyzable, e.g by tyrosine phosphatases. Inclusion of a nonhydrolyzable phosphotyrosine analog allows the peptides of the invention to

retain binding and/or inhibitory activity for longer periods of time, in the presence of agents which may remove the phosphate group from the phosphotyrosine, *e.g.*, tyrosine phosphatases, thereby allowing for more effective inhibition and reduced effective amounts, among other benefits. Examples of phosphotyrosine analogs having nonhydrolyzable phosphate groups include, *e.g.*, (phosphonomethyl)phenylalanine ("Pmp"). Pmp is a phosphotyrosine analog in which the $>C\text{-}O\text{-}PO_3H_2$ group of pY has been replaced by $>C\text{-}CH_2\text{-}PO_3H_2$. Inclusion of this analog within sequences recognized by other phosphotyrosine binding domains yields comparable binding as with their phosphotyrosine-containing counterparts. *See*, Domchek, et al., Biochem. (1992) 31:9865-9870. Thus, in an aspect of the present invention, the peptides of the present invention which comprise a core sequence ~~$NX_3X_1X_2X_4$~~ $NX_3X_1X_2X_4$ (SEQ ID NO:1), where ~~X_1, X_2, X_3 and X_4~~ X_1, X_2, X_3 and X_4 are as previously described, the phosphotyrosine residues in X_1 and/or X_2 are substituted with Pmp.

Paragraph beginning at page 33, line 19, has been amended as follows:

The direct binding of the phosphorylated peptide PAFSPAFDNL(pY)(pY)WDQNSSEQG ("b-phos.") (SEQ ID NO:10) to the PTB domain is shown in Figure 3. This peptide bound the PTB domain both in the presence and absence of a 100X concentration of unphosphorylated, non-biotinylated peptide. PTB binding was inhibited in the presence of 100X concentration of phosphorylated peptide, which competed for the PTB domain. Unphosphorylated, biotinylated peptide did not bind the PTB domain. Neither the phosphorylated nor unphosphorylated form of this peptide were able to specifically bind to an SH2 domain.

Paragraph beginning at page 33, line 30, has been amended as follows:

The peptides PAFSPAFDQL(pY)(pY)WDQNSSEQG ("b-N1219Q") (SEQ ID NO:72) and PAFSPAFDDL(pY)(pY)WDQNSSEQG ("b-N1219D") (SEQ ID NO:73) which

carried point mutations in the asparagine residue in the ninth position, also show substantially reduced binding to the PTB domain in these assays (Figure 3).

IN THE CLAIMS:

The claims have been amended as follows:

1. (Amended) A substantially pure peptide which is capable of binding a PTB domain, wherein the peptide is from 5 to 100 amino acids in length, and comprises a core sequence of aminoacids $NX_3X_1X_2X_4$;

wherein X_1 is selected from the group consisting of Y, pY or an analog thereof, E, T, D, Q, A and F;

X_2 is selected from pY or an analog thereof, and Y, provided that at least one of X_1 and X_2 is pY, or an analog thereof;

X_3 is selected from the group consisting of L and A; and

X4 is selected from the group consisting of W, L, S, F and Q (SEQ ID No:1).

2. (Amended) The peptide as recited in claim 1, wherein the peptide is from 6 to 100 amino acids in length, and comprises a core sequence of amino acids $X_5NX_3X_1X_2X_4$, wherein X_5 is selected from the group consisting of D, S, E and A (SEQ ID NO:2).

4. (Amended) The peptide as recited in claim 3, wherein the peptide is from 6 to 100 amino acids in length,. and comprises a core sequence of amino acids selected from the group consisting of $DNX_3X_1pYX_4$ (SEQ ID NO:3) and $ENX_3X_1PYX_4$ (SEQ ID NO:4), where X4 is selected from the group consisting of W and F.

5. (Amended) The peptide as recited in claim 2, wherein the peptide is from 12 to 100 amino acids in length, and comprises a core sequence of amino acids selected from the group consisting of AFDNLY(pY)WDQNS (SEQ ID NO:5), AFDNL(PY)YWDQNS (SEQ ID NO:6) and AFDNL(pY)(pY)WDQNS (SEQ ID NO:7).

6. (Amended) The peptide as recited in claim 2, wherein the peptide is from 21 to 100 amino acids in length, and comprises a core sequence of amino acids selected from the group consisting of: PAFSPAFDNLY(pY)WDQNSSEQG (SEQ ID NO:8); PAFSPAFDNL(pY)YWDQNSSEQG (SEQ ID NO:9); PAFSPAFDNL(pY)(pY)WDQNSSEQG (SEQ ID NO:10); PAFSPAADNLY(pY)WDQNSSEQG (SEQ ID NO:ll); PAFSPAADNL(pY)YWDQNSSEQG (SEQ ID NO:12); PAFSPAADNL(pY)(pY)WDQNSSEQG (SEQ ID NO:13); PAFSPAFANLY(pY)WDQNSSEQG (SEQ ID NO:14); PAFSPAFANL(pY)YWDQNSSEQG (SEQ ID NO:15); PAFSPAFANL(pY)(pY)WDQNSSEQG (SEQ ID NO:16); PAFSPAFSNLY(pY)WDQNSSEQG (SEQ ID NO:17); PAFSPAFSNL(pY)YWDQNSSEQG (SEQ ID NO:18); PAFSPAFSNL(pY)(pY)WDQNSSEQG (SEQ ID NO:19); PAFSPAFDNAY(pY)WDQNSSEQG (SEQ ID NO:20); PAFSPAFDNA(pY)YWDQNSSEQG (SEQ ID NO:21); PAFSPAFDNA(pY)(pY)WDQNSSEQG (SEQ ID-NO:22); PAFSPAFDNLA(pY)WDQNSSEQG (SEQ ID NO:23); PAFSPAFDNLF(pY)WDQNSSEQG (SEQ ID NO:24); PAFSPAFDNLY(pY)FDQNSSEQG (SEQ ID NO:25); PAFSPAFDNL(pY)YFDQNSSEQG (SEQ ID NO:26); PAFSPAFDNL(pY)(pY)FDQNSSEQG (SEQ ID NO:27); PAFSPAFDNLY(pY)WAQNSSEQG (SEQ ID NO:28); PAFSPAFDNL(pY)YWAQNSSEQG (SEQ ID NO:29); PAFSPAFDNL(pY)(pY)WAQNSSEQG (SEQ ID NO:30); PAFSPAFDNLY(pY)WDANSSEQG (SEQ ID NO:31); PAFSPAFDNL(pY)YWDANSSEQG (SEQ ID NO:32); PAFSPAFDNL(pY)(pY)WDANSSEQG (SEQ ID NO:33); PAFSPAFDNLY(pY)WDNNSSEQG (SEQ ID NO:34); PAFSPAFDNL(pY)YWDNNSSEQG (SEQ ID NO:35); PAFSPAFDNL(pY)(pY)WDNNSSEQG (SEQ ID NO:36); PAFSPAFDNLY(pY)WDDNSSEQG (SEQ ID NO:37); PAFSPAFDNL(pY)YWDDNSSEQG (SEQ ID NO:38); PAFSPAFDNL(pY)(pY)WDDNSSEQG (SEQ ID NO:39); PAFSPAFDNLY(pY)WDQASSEQG (SEQ ID NO:40); PAFSPAFDNL(PY)YWDQASSEQG (SEQ ID NO:41); PAFSPAFDNL(pY)(pY)WDQASSEQG (SEQ ID NO:42); PAFSPAFDNLY(pY)WDQNASEQG (SEQ ID NO:43);

PAFSPAFDNL(pY)YWDQNASEQG (SEQ ID NO:44); and
PAFSPAFDNL(pY)(pY)WDQNASEQG (SEQ ID NO:45).

8. (Amended) A substantially pure peptide which is capable of binding a PTB domain, wherein the peptide is from 21 to about loo amino acids in length and which comprises a core sequence of amino acids selected from the group consisting of AFGGAVENPE(pY)LAPRAGTASQ (SEQ ID NO:46) and EGTPTAENPE(pY)LGLDVPV (SEQ ID NO:47).

10. (Amended) A method of determining whether a protein comprises a PTB domain, comprising the steps of:

contacting the protein with a peptide, which peptide is from 5 to 100 amino acids in length and comprises a core sequence of amino acids $NX_3X_1X_2X_4$, wherein X_1 is selected from the group consisting of Y, pY, E, T, D, Q, A and F; X_2 is selected from pY and Y, provided that at least one of X_1 and X_2 is pY; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID NO:l); and determining whether the peptide binds to the protein during said contacting step, where the binding of the peptide to the protein is indicative that the protein comprises a PTB domain.

13. (Amended) A method of determining whether a test compound is an agonist or antagonist of a PTB/phosphorylated ligand interaction, comprising the steps of:

incubating the test compound with a protein comprising a PTB domain and a peptide, which peptide is from 5 to 100 amino acids in length and which comprises a core amino acid sequence $NX_3X_1X_2X_4$, wherein X_1 is selected f rom the group consisting of Y, pY, E, T, D, Q, A and F; X_2 is selected from pY and Y, provided that at least one of X_1 and X_2 is pY; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID NO:l); and

determining the amount of protein bound to the peptide during said incubating step; and

comparing the amount of protein bound to the peptide during said incubating step to an amount of protein bound to the peptide in the absence of the test compound, the increase or

decrease in the amount of protein bound to the peptide in the presence of the test compound being indicative that the test compound is an agonist or antagonist of PTB domain/phosphorylate ligand interaction, respectively.

17. (Amended) A method of obtaining substantially pure PTB-domain-containing protein from a mixture of different proteins, comprising the steps of:

providing a peptide which is from 5 to 100 amino acids in length, and which comprises a core amino acid sequence $NX_3X_1X_2X_4$, wherein X_1 is selected from the group consisting of Y, pY, E, T, D, Q, A and F; X_2 is selected from pY and Y, provided that at least one of X_1 and X_2 is pY; X_3 is selected from the group consisting of L and A; and X_4 is selected from the group consisting of W, L, S, F and Q (SEQ ID-NO:l); bound to a solid support;

contacting the mixture of different proteins with the peptide bound to the solid support whereby the PTB domain-containing protein is bound to the peptide;

washing the solid support to remove unbound proteins;

and

eluting substantially pure PTB-domain-containing protein from the solid support.